STAINING METHODS IN BACTERIOLOGY

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Introduction

-Stained preparations are needed to examine micro-organisms microscopically in order to study their morphology and observe their cellular components.

-Smears are made and stained by any one of the required staining methods.

Smear Preparation

The process of making a smear preparation is an important skill in the microbiology laboratory and is usually the first step in most staining procedures.

Instructions

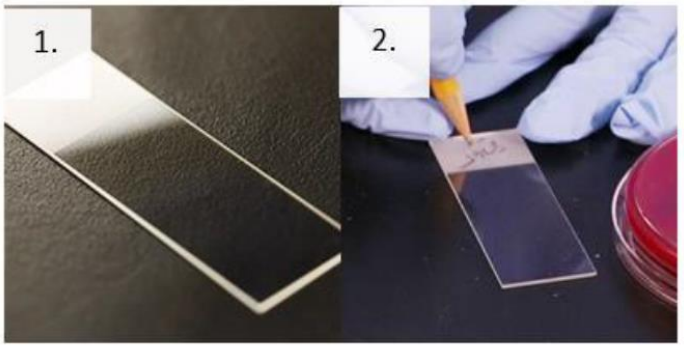
1. Obtain a clean microscope slide with a frosted edge.
2. Label the frosted edge with the sample identification.
3. Transfer specimen or culture to the center of the slide.

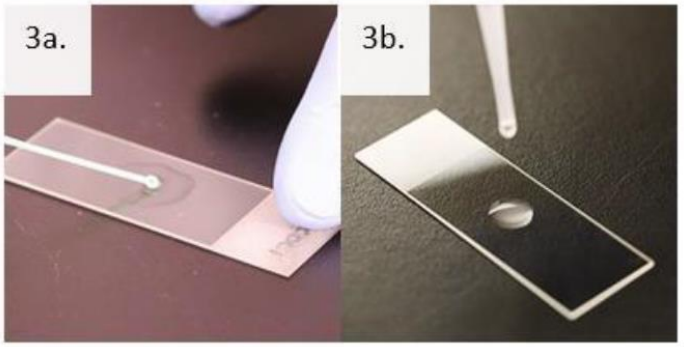
a. Clinical Specimen: Prepare a thin layer of cells on the slide.

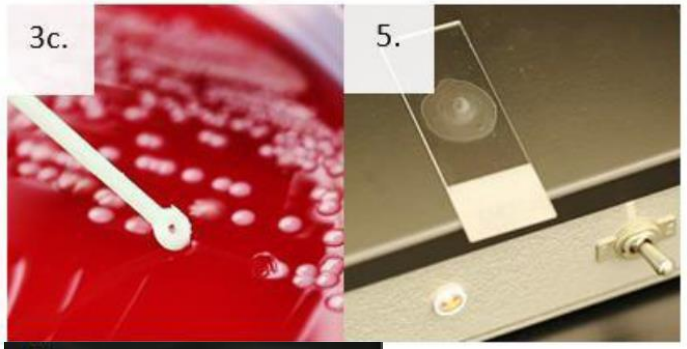
b. Broth Culture: Using a sterile pipette, transfer 1-2 drops to the slide. Spread the drop into a thin, even smear.

c. Culture from solid media: Using a sterile pipette, add one drop of sterile saline or sterile water to the center of the microscope slide. Aseptically pick a small amount of an isolated colony with a loop and gently mix into the drop of sterile saline or water using circular motions. Mix evenly to make a thin smear.

5. Allow the smear to air dry completely.
6. Fix the smear to the slide using heat fixation.
7. Allow the slide to cool to room temperature or air dry.

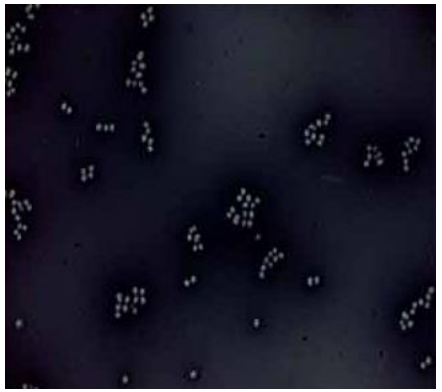






Staining:-

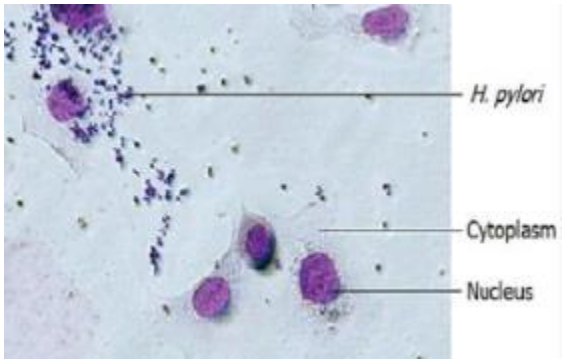
Stains are colored organic compounds used for staining microorganism, it can classified according to nature of stain:



1-Acidic dyes:- These contain functional groups that have a negative charge. Examples include eosin, nigrosin and Congo red. These dyes are repelled by the negatively charged surface of bacterial cells. Thus, they stain the background, leaving the bacterial cells clear and bright against a dark background.

2-Basic dyes:- These dyes contain positively charged groups. Examples include methylene blue, basic fuchsin, and crystal violet. These dyes directly bind to and stain the negatively charged surface of bacterial cells.

3-Neutral dyes:- These dyes are formed by the combination of acidic and basic stains in aqueous form. They stain cytoplasm and nucleic acid , for example Giemsa stain.



Three Types of Staining Procedures:

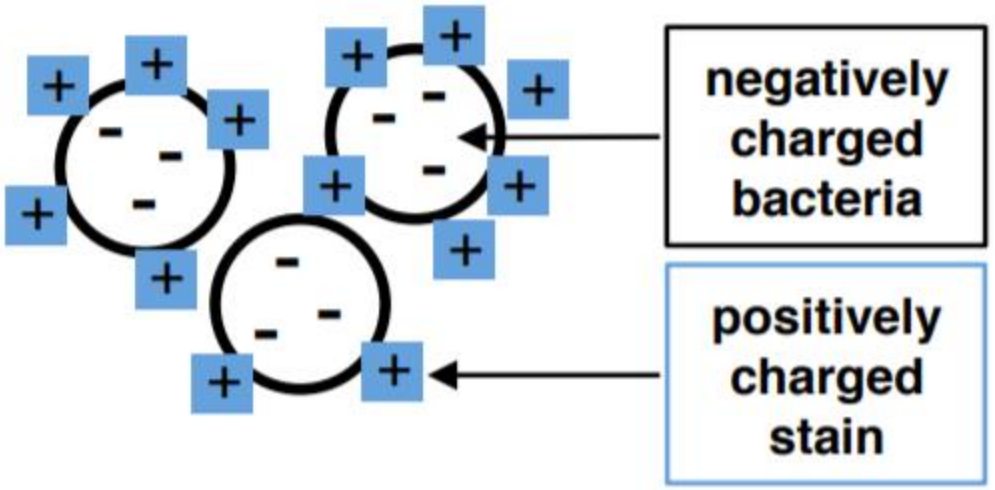
1-Simple Staining use only one dye to detect shapes and arrangements.

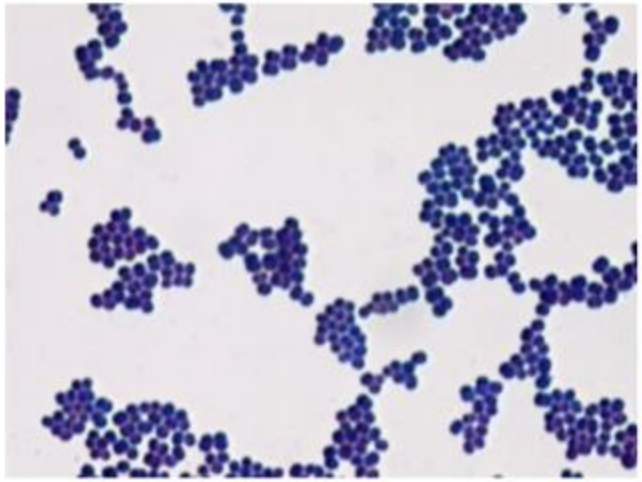
2-Differential Staining (Gram stain, Ziel-Neelsen stain).

3-Special Staining (Capsule, flagella, spores).

1-Simple stain

Methylene blue is a simple and direct stain used for determining bacterial morphology (shape and arrangement). It is a basic dye (positive charge) which stains the cell a blue color. The presence of negatively charged molecules in the cell causes the cell to stain blue.

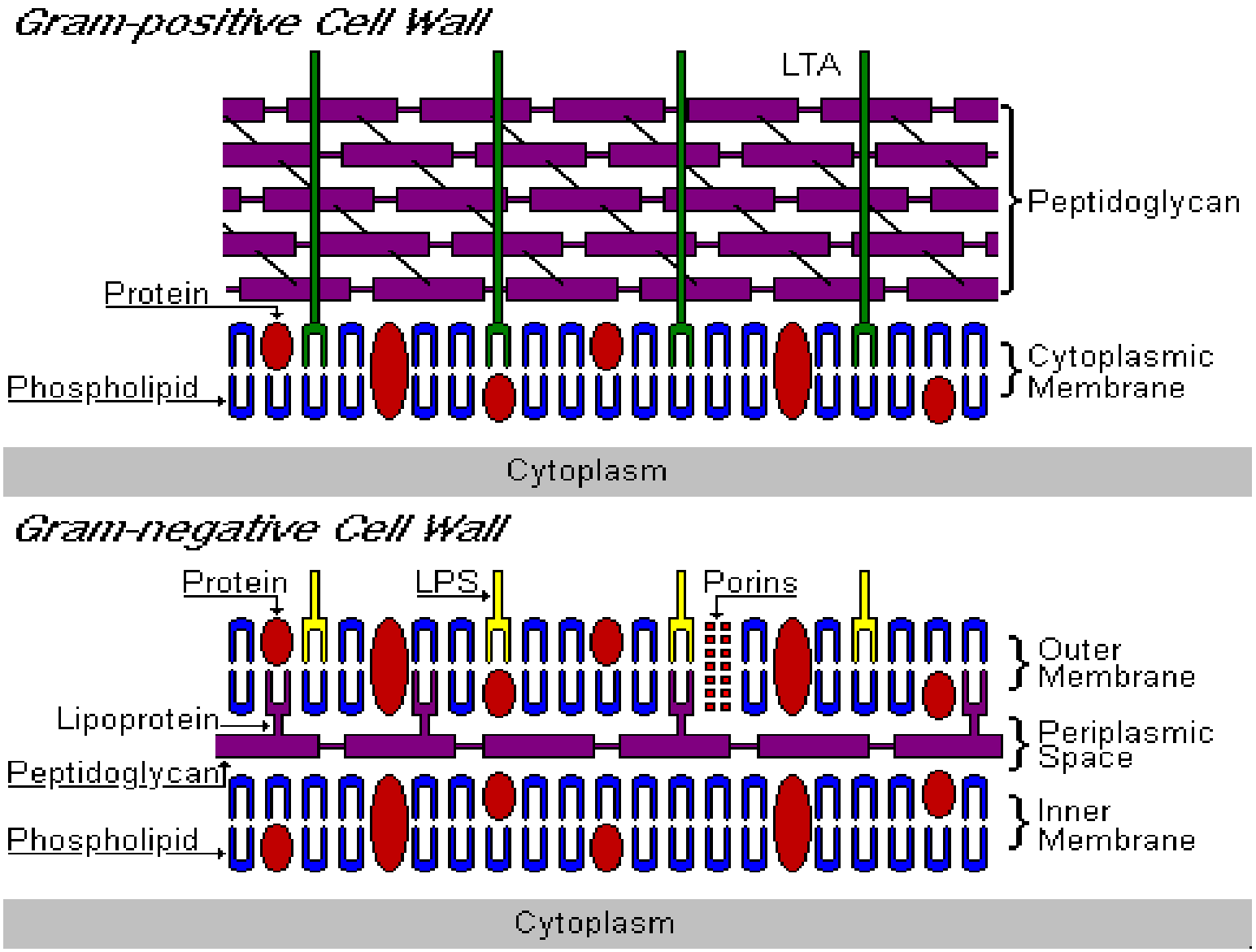




2-Gram staining

Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall components.

Gram staining involves three processes: staining with a water-soluble dye called **crystal violet, decolorization**, and counterstaining, usually with **safranin**. Due to differences in the thickness of a peptidoglycan layer in the cell membrane between Gram positive and Gram negative bacteria, Gram positive bacteria (with a thicker peptidoglycan layer) retain crystal violet stain during the decolorization process, while Gram negative bacteria lose the crystal violet stain and are instead stained by the safranin in the final staining process.


Gram-positive Cell Wall
LTA
Peptidoglycan
Protein
Phospholipid
Cytoplasmic Membrane
Cytoplasm
Gram-negative Cell Wall
Protein
LPS
Porins
Outer Membrane
Lipoprotein
Periplasmic Space
Peptidoglycan
Phospholipid
Inner Membrane
Cytoplasm

Staining mechanism

There are four basic steps of the Gram stain

1-Crystal Violet : Crystal violet + ion will penetrate through the cell wall and cell membrane of both gram-positive and gram-negative cells, The CV+ion interacts with negatively charged components of bacterial cells. It is act as **primary stain.**

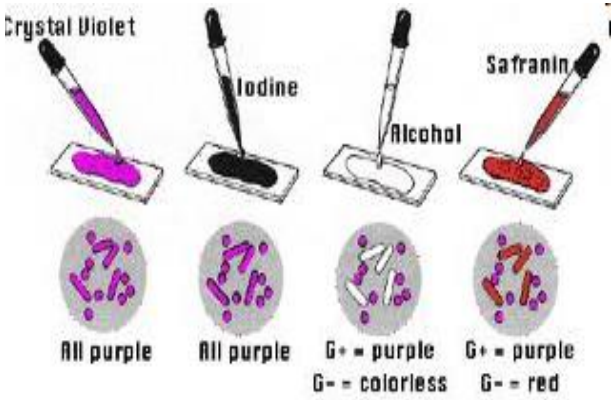
2-Iodide (I) interacts with CV+ and forms large complexes (CV–I) within the inner and outer layers of the cell. Iodine is often referred to as a **mordant.**

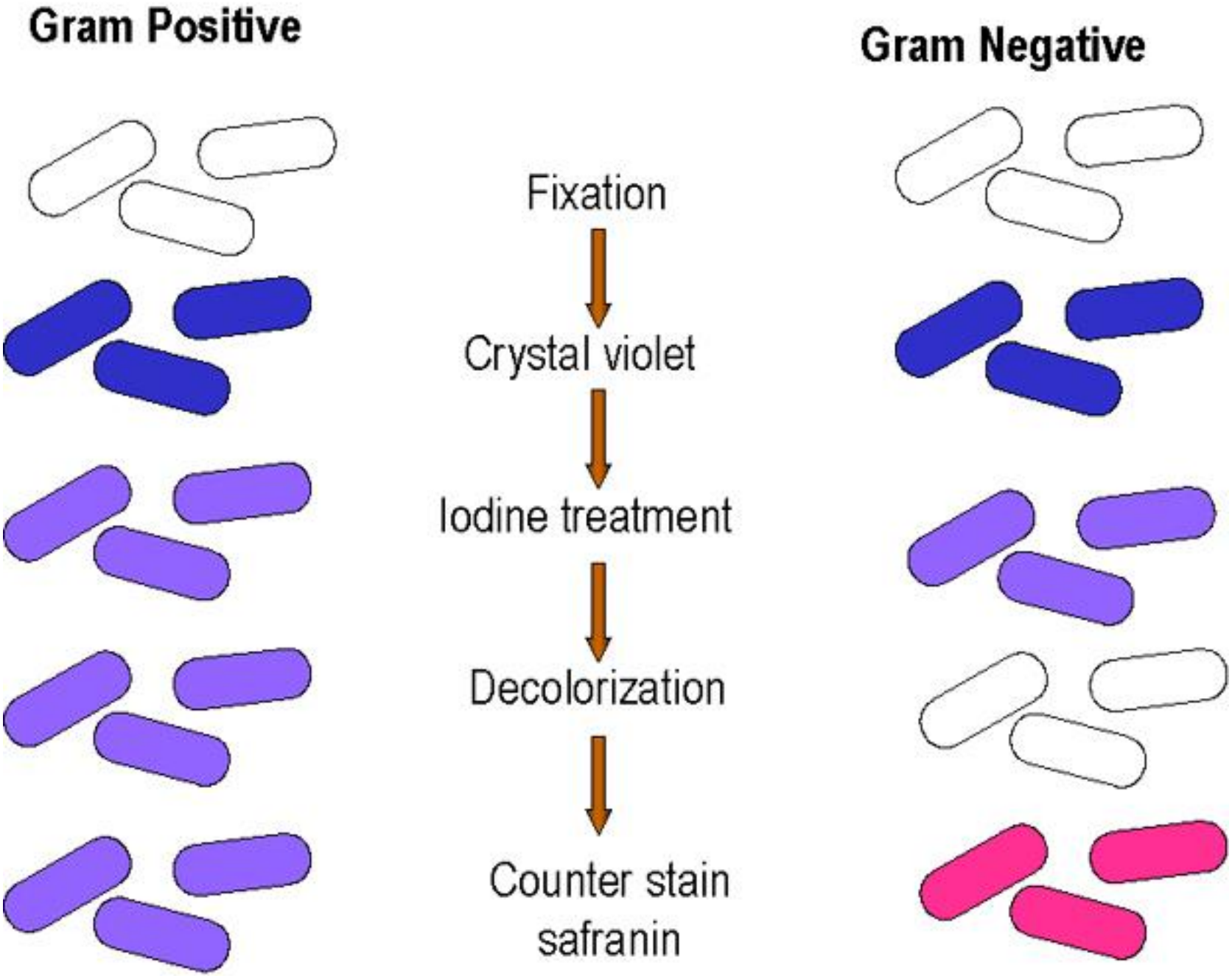
3-Alcohol used as **decolorizer**, it interacts with the lipids of the cell membrane. A gram-negative cell loses its outer lipopolysaccharide membrane, and the inner peptidoglycan layer is left exposed. The CV–I complexes are washed from the gram-negative cell along with the outer membrane. In contrast, the large CV–I complexes become trapped within the gram-positive cell due to the multilayered nature of its peptidoglycan.

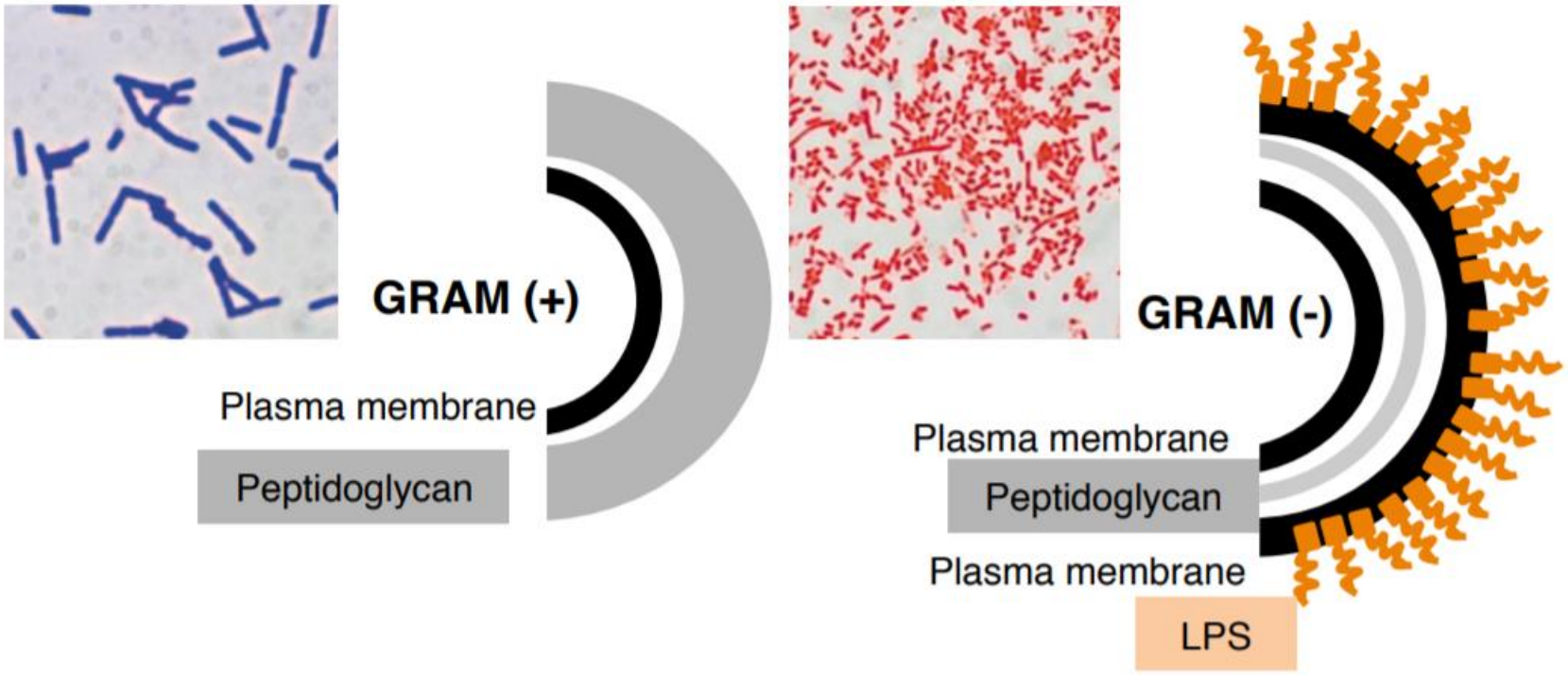
4- Safranin used as **counter stain**, after decolorization, the gram-positive cell remains purple and the gram-negative cell loses its purple color and take a pink or red color.

GRAM STAINING PROCEDURE

- Prepare a heat fixed bacterial smear.
- Flood the smear with crystal violet (1to 2 min)
- Quickly and gently wash off excess stain (2 seconds)
- Flood the smear with Grams iodine (1 minute)
- Decolorize with alcohol (10-20 seconds or until the excess alcohol which flow off the slide is colorless)
- Quickly and gently wash off excess stain (2 seconds)
- Flood the smear with safranin (30 sec to 2 min.)
- Quickly and gently wash off excess stain (2 seconds)
- Blot dry with bibulous paper
- Examine your slide under the microscope.




Gram Positive
Gram Negative
Fixation
Crystal violet
Iodine treatment
Decolorization
Counter stain
safranin


GRAM (+)
Plasma membrane
Peptidoglycan
GRAM (-)
Plasma membrane
Peptidoglycan
Plasma membrane
LPS

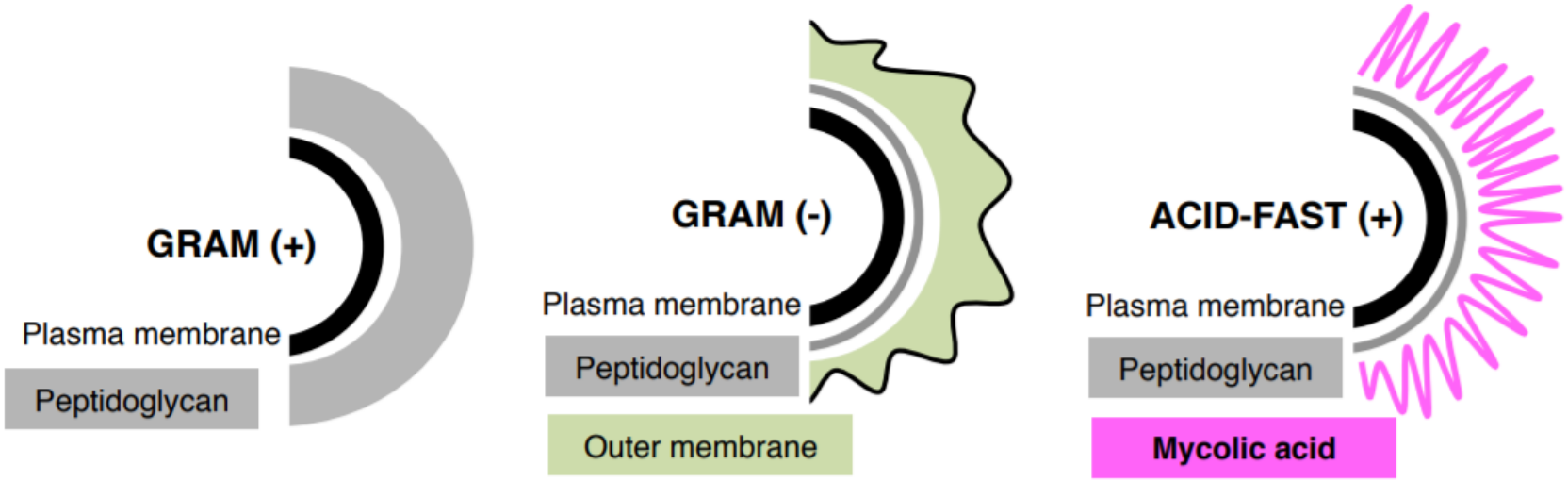
Acid Fast Staining (Ziehl-Neelsen stain)

Principle

- *Mycobacterium* and many *Nocardia* species are called **acid-fast** because during an acid-fast staining procedure they retain the primary dye carbol fuchsin despite decolorization with the powerful solvent **acid-alcohol**. Nearly all other genera of bacteria are **nonacid-fast**.
- They contain a waxy lipid, mycolic acid in their cell wall. Acid fast cell walls are so durable that the stain (Carbol Fuchsin) must be driven into the cells by heat. The cells are then decolorized with acid-alcohol, all other cells will decolorize with this strong solvent, but acid fast bacteria will not. Other cells are then counter stained with Methylene blue.

Material

- Carbol Fuchsin (3-5 min) with heat
- Acide- alcohol(2.5% HCL in ethanol) for (10-30 sec).
- Methylene blue(2 min).



Mycobacterium	non-myco	
		1. All bacteria are transparent prior to staining
		2. Steam is used to force the CARBOL FUCHSIN into all the bacteria
		3. Decolorization with acid removes the CARBOL FUCHSIN from the bacteria that do not have mycolic acid
		4. Methylene blue is the counterstain

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2

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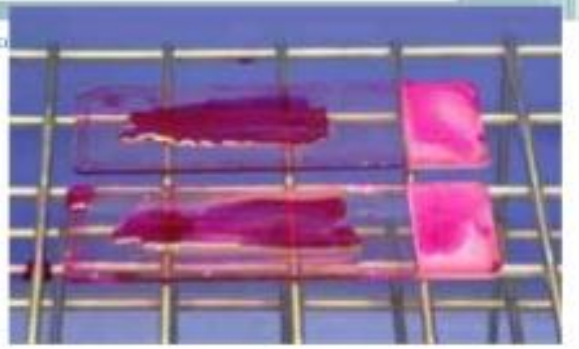

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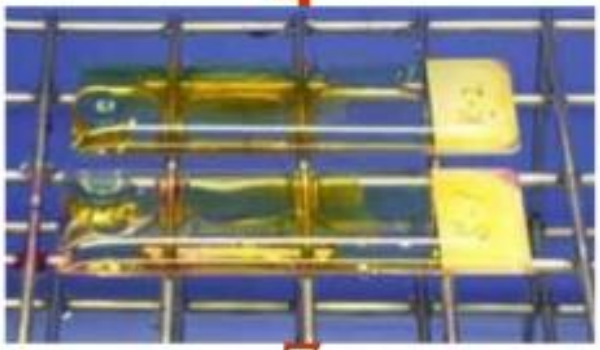

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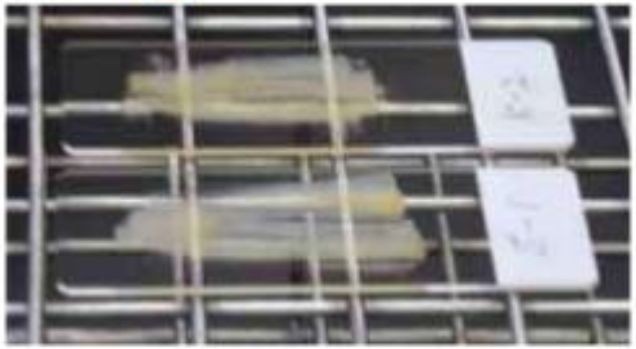

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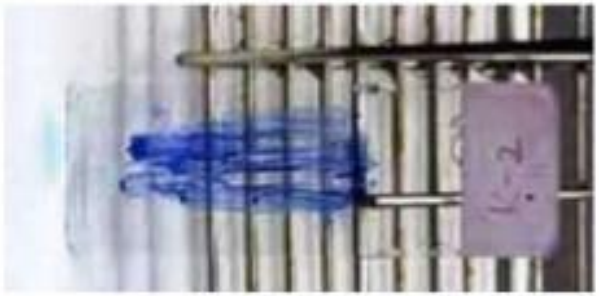
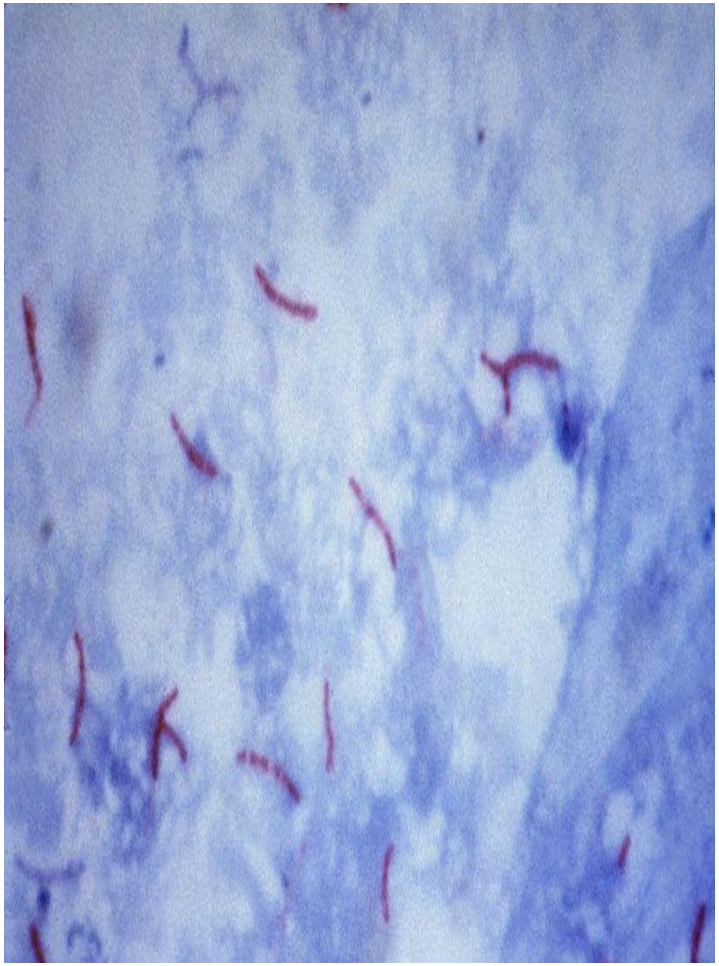


Ziehl-Neelsen stain



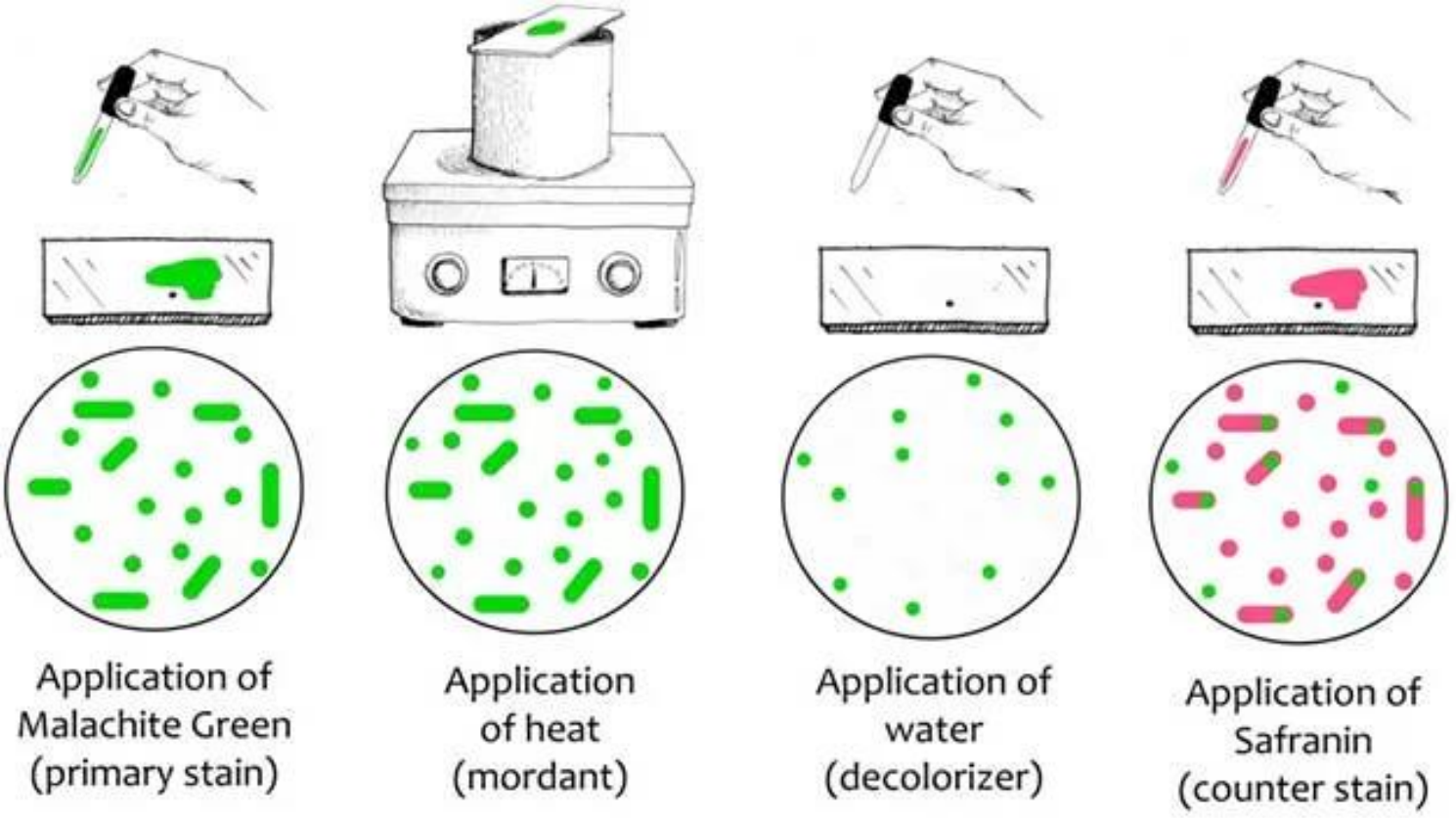
M. Tuberculosis (pink bacilli).

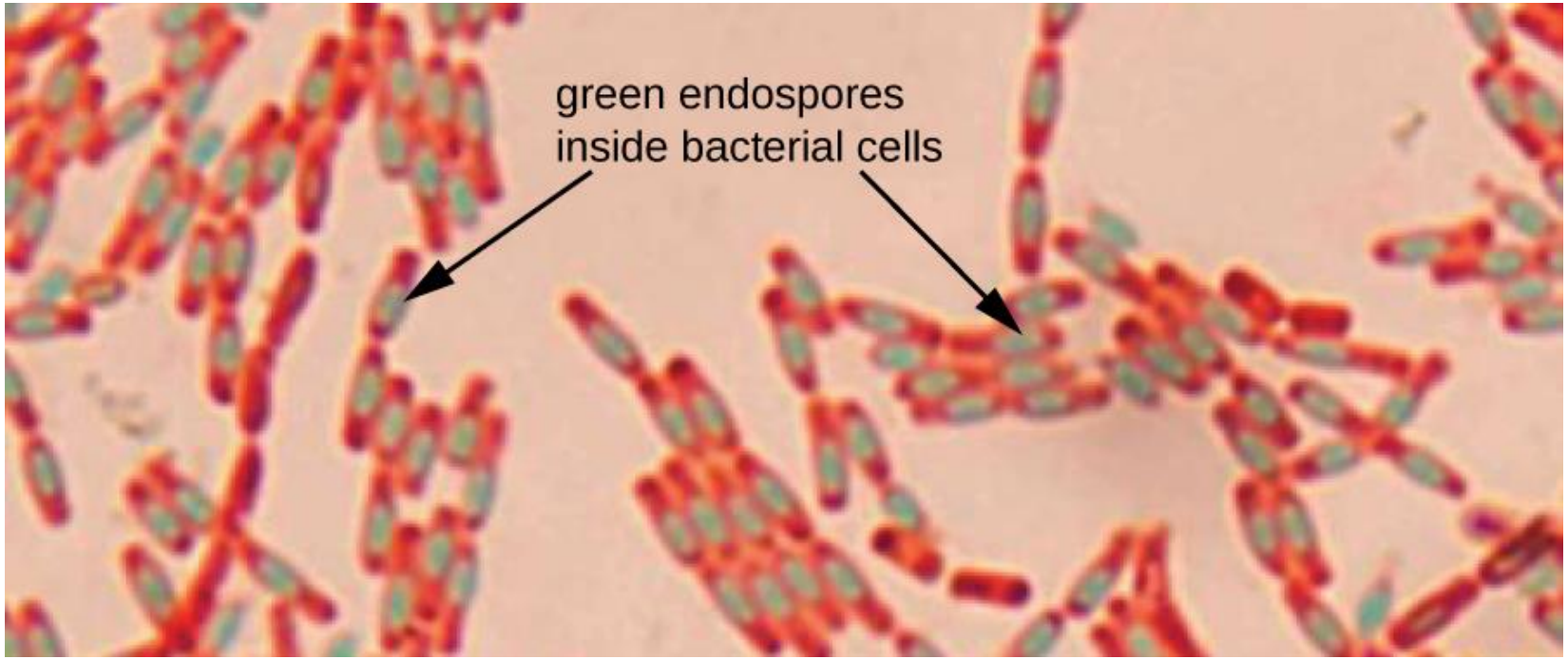
Special Staining

-Spore stain

Malachite green **(primary dye)** is used with heat to force the stain into the cells and give them color. The heating of the bacteria will make the spore wall more permeable to the malachite green, and it then attaches to the peptidoglycan.

A counter stain, safranin, is then used to give color to the non spore forming bacteria. At the end of the procedure, spores stain green and other cells stain red.


Application of Malachite Green (primary stain)
Application of heat (mordant)
Application of water (decolorizer)
Application of Safranin (counter stain)


green endospores
inside bacterial cells

THANK YOU